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The evolution of conflict resolution between plasmids and their bacterial hosts

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Plasmid-host genetic conflict

Summary

It has recently been proposed that mobile elements may be a significant driver of cooperation in microorganisms. This may drive a potential conflict, where cooperative genes are transmitted independently of the rest of the genome, resulting in scenarios where horizontally spread cooperative genes are favoured while a chromosomal equivalent would not be. This can lead to the whole genome being exploited by surrounding non-cooperative individuals. Given that there are costs associated with mobile elements themselves, infection with a plasmid carrying a cooperative trait may lead to a significant conflict within the host genome. Here we model the mechanisms that allow the host to resolve this conflict, either by exhibiting complete resistance to the mobile element or by controlling its gene expression via a chromosomally-based suppressor. We find that the gene suppression mechanism will be more stable than full resistance, implying that suppressing the expression of costly genes within a cell is preferable to preventing the acquisition of the mobile element, for the resolution of conflict within a genome.

Key words

Resistance, selfish genetic elements, horizontal gene transfer, coevolution

Introduction

While genomes are often seen as structured entities working towards the same goal, conflict beneath the surface is in fact ubiquitous (Keller 1999; Burt and Trivers 2008). Conflict arises when individual genetic entities, be they individual genes, chromosomes or foreign DNA, such as phage or plasmids, act to maximize their own fitness at the expense of the genome as

a whole. In the latter case, the genomic conflict refers to conflict between disparate genetic elements within a single cell. Plasmids are extra-chromosomal pieces of DNA which use the cell's machinery to replicate independently of the host genome (Novick 1987; Sota and Top 2008). They can be viewed as genomic parasites (Simonsen 1991), as they often code for genes which are advantageous to their own persistence and transmission, but disadvantageous to the host cell (Rankin et al. 2011b), for example, in the case of toxin anti-toxin complexes (Jensen and Gerdes 1995; Holcik and Iyer 1997; Hayes 2003). In addition, there are many costs associated with plasmid carriage (Dahlberg and Chao 2003; Wagner and Hewlett 2004; Lili et al. 2007) that lead to frequent conflict between the plasmid's replication and the survival of its host. In this sense plasmids are molecular parasites, and one may expect coevolution between the bacterial and plasmid chromosomes to facilitate a reduction in the deleterious effect of the plasmid (Bouma and Lenski 1988; Modi and Adams 1991).

Plasmids code for a diverse array of traits (Schumann 2001), but in particular it has been shown that they carry a disproportionate amount of genes involved in bacterial virulence and cooperation (Nogueira et al. 2009), suggesting a key role for plasmids in bacterial social evolution. Such traits include those which detoxify the local environment (e.g. Lee et al. 2006; Ellis et al. 2007); are involved in communication (e.g. Gonzalez and Marketon 2003; Penalver et al. 2006); and toxins (e.g. Ahmer et al. 1999). It is well established that a cooperative gene can invade from rare in a population if $BR>C$, where B is the benefit to producing a public good, C is the cost and R is the genetic relatedness (Hamilton 1964). It has been shown that relatedness can act to promote cooperation in the production of microbial public goods (Griffin et al. 2004). Plasmids have been found to be important vectors of cooperative genes (Nogueira et al. 2009). If a gene coding for cooperation is carried on a plasmid, then such a gene will have two advantages (Smith 2001; Nogueira et al. 2009; Mc Ginty et al. 2011; Rankin et al. 2011a): the ability to spread by infecting nearby cells and an increase in relatedness between other neighbouring individuals as a result of infection (since relatedness is measured at the locus of interest, which in this case is a gene on a plasmid –

Nogueira et al. 2009). Thus, the condition for a plasmid to spread from rare could be written as a modified form of Hamilton's rule, where invasion is boosted by both additional infectivity and relatedness is amplified by horizontal transfer i.e. $B(R+f)+g>C$, where f refers to the amplification of the plasmid on within-group relatedness, while g refers to the benefit to the plasmid from spreading to new cells. Both f and g would depend on factors such as the probability of transmission to an infected cell, the proportion of infected cells and the number of initial strains in a local neighbourhood. From this extended Hamilton's rule, it is evident that a plasmid carrying a gene for cooperation can spread even if $BR<C$, as it can mitigate the cost of cooperation by amplifying relatedness (through a higher f) and transmitting to other cells (through a higher g). Under this condition, there is the potential for a conflict to exist between the chromosome and a plasmid, whenever there is a net cost of a gene carried on the chromosome (i.e. if $BR<C$) but a net benefit of that same gene carried on a conjugative plasmid (i.e. if $B(R+f)+g>C$). Following the notation above, such conflict will occur if $B(R+f)+g>C>BR$, which is referred to as the "conflict zone".

A gene on a chromosome that actively suppresses expression of the cooperative gene, or prevents itself from being infected by a plasmid (which also precludes the costs associated with plasmid carriage itself), will confer an advantage over one that retains the plasmid and allows expression of the cooperative gene. As it has been shown that cooperative genes are over-represented on plasmids (Nogueira et al. 2009), it is likely that cooperative genes carried

on plasmids are favoured in a way that chromosomally-carried versions are not, suggesting that conflict between plasmid-carried cooperative genes and the host chromosome may be common. As such, we would expect mechanisms from the host genome to resist the costs of cooperative plasmids which consist of both the cost of plasmid carriage and of cooperation behaviour (Bouma and Lenski 1988; Modi and Adams 1991).

A variety of mechanisms exist to defend against mobile elements (Johnson 2007) which have the potential to resolve this conflict. Here we focus on two such mechanisms: one targeting the plasmid itself and the other the cooperative gene. The first mechanism we explore is direct resistance to plasmid infection, that is, carriage of a trait that prevents conjugation or breaks down foreign DNA. This occurs in the case of restriction-modification systems (Levin 1993; Stern and Sorek 2011), or CRISPR-Cas systems (van der Oost et al. 2009; Vale and Little 2010), which act as a bacterial immune system, protecting the cell against foreign DNA. The second mechanism we examine sees the conflict resolved through a host allele which suppresses expression of the plasmid-carried gene. Such gene interactions, often involving plasmids and phages, are common in bacteria (Chernin and Mikoyan 1981; Close et al. 1985; Harr and Schlotterer 2006; Cámara et al. 2010; Gordo and Sousa 2010; Shintani et al. 2010). Here we examine the evolution of a chromosomal gene which suppresses the expression of the plasmid gene coding for the public good. We then compare the evolutionary conditions that favour these two mechanisms to look at the evolution, and the resolution, of a conflict between plasmids and their bacterial hosts.

Model and Results

Model Structure

We use the neighbour-modulated fitness approach (Taylor and Frank 1996; Frank 1998; West and Buckling 2003; Gardner et al. 2004) to model a large population of host-associated bacteria which are subdivided into an infinite number of hosts which we refer to herein as "patches". Our life cycle consists of five stages. (1) Founding: Each patch is initially infected with an inoculum of N founder strains randomly sampled from an infinite pool of potential founder strains. We assume that, at this stage, all cells are capable of reproducing. (2) Proliferation: Founder cells divide and grow to a large number within the patch, meaning that (in the absence of transmission) the whole-group relatedness is $R=1/N$. Founder cells die after reproduction. Bacteria may be plasmid-carriers or plasmid-free and plasmids are inherited vertically during cell division. As the rate of segregation is generally on the order of 10^{-6} or lower (Simonsen 1991), we assume that segregation is negligible, and do not include it in our models. (3) Horizontal gene transfer (HGT): We model transmission using the probability β, which is the probability that, given that an uninfected cell meets an infected cell, the plasmid will be transmitted. For simplicity we assume that only uninfected cells can acquire a plasmid during this stage and conjugation therefore requires an uninfected cell to make contact with an infected cell, before conjugation can occur. (4) Fitness Interaction: Offspring survival is determined by results of interaction between cells based on the fitness function described below. (5) Dispersal: Any associations between the two loci are disrupted by recombination and all descendant bacterial cells compete globally to found new patches. Unsuccessful bacteria (i.e. those that fail to infect a new patch) die. Every time step patches are cleared of all bacteria (either immune clearance or patch extinction/host death), and the lifecycle begins again.

We begin with the Price equation (Price 1970, 1972), which describes the change in gene frequency for a given gene, and allows us to partition the effects of both selection and and transmission on the change in gene frequency:

$$\underbrace{\Delta p_x}_{\text{Change in gene frequency}} = \underbrace{\frac{1}{w}\text{Cov}[w_{ij}, p_{xij}]}_{\text{Selection}} + \underbrace{\frac{1}{w}\text{E}[w_{ij}, \Delta p_{xij}]}_{\text{Transmission}} \quad [1]$$

where w_{ij} is the fitness of individual i in patch j, p_{xij} is an indicator variable describing whether individual i in patch j carries trait x (i.e. the plasmid or the resistance trait, taking a value of one if it has the given gene and zero if it doesn't), w_{ij} and p_{xij} are random variables and w and p_x are, respectively, the mean fitness and mean frequency of carriers of trait x across the whole population after transmission and selection. In equation (1) (and all following analyses), both the covariance term and expectation term are taken over all individuals (i) in all patches (j).

Model 1 –Cooperative plasmids and chromosomal resistance

We start by investigating the condition for a plasmid which carries a gene for cooperation to invade. We denote p_1 as the global plasmid frequency among the founding innoculum, and the frequency after the transmission stage is denoted p_1^t (where the superscript denotes that p_1 is measured after transmission). The plasmid comes at a cost C_1 to the host cell, and confers a benefit B on other cells in the local environment. C_1 consists of a baseline cost of carriage (v) in addition to a cost for expression of social behavior (C) (so that $C_1=C+v$). We assume that some cells carry a gene which confers resistance to a plasmid, and thus cannot be infected by a plasmid. The global frequency of such resistant cells is denoted by p_2. Such resistance incurs a cost C_2 to the resistant individual and can be seen as a form of bacterial immune system, which would protect the cell against foreign DNA, such as plasmids. For example, this could come either in the form of a CRISPR-Cas system, where an infecting plasmid would be removed from the bacterial host (Sorek et al. 2008; van der Oost et al. 2009; Vale and Little 2010) or a restriction modification system whereby foreign DNA is degraded by a restriction endonuclease (Wilson and Murray 1991; Stern and Sorek 2011). As we assume full effectiveness of the resistance gene, individuals who carry p_2 cannot carry p_1 and vice versa. Individual fitness after transmission and interaction is therefore:

$$w_{ij} = 1 - C_1 p_{1ij}{}^{t}(1 - p_{2ij}{}^{t}) - C_2 p_{2ij}{}^{t}(1 - p_{1ij}{}^{t}) + B p_{1j}{}^{t}. \quad [2]$$

The terms used to calculate fitness are listed in Table 1. The average frequency of the plasmid after transmission (p_1^t), is the sum of those cells who carried the plasmid at the beginning of the life cycle (p_1) plus those plasmid-free cells which were infected with the plasmid at the transmission stage (as they also do not carry the resistance trait) such that $p_1^{\ t} = p_1 + (1 - p_1 - p_2)\frac{N-1}{N}\beta p_1$. The frequency of resistance gene after transmission is denoted as $p_2^{\ t}$ (where $p_2^{\ t} = p_2$), since resistant individuals are not affected by plasmid transmission.

Table 1. List of terms used to generate fitness function *w*. All terms refer to both resistance and suppressor models unless otherwise stated.

Term	Definition
w_{ij}	Fitness of an individual *i* in patch *j*. This is a random variable.
w	Global mean fitness across all individuals in all patches in the population such that $w = \mathrm{E}[w_{ij}] = \sum_{ij} w_{ij}/(Nn)$.
p_{xij}	An indicator variable that takes the value 1 if the individual carries allele *x* and 0 otherwise. This is a random variable. A superscript *t* indicates when this is measured after transmission.
p_x	Global mean frequency of allele *x* across all individuals in all patches in the population such that $p_x = \mathrm{E}[p_{xij}] = \lim_{n\to\infty}\left[\sum_{ij} p_{xij}/(Nn)\right]$. A superscript *t* indicates when this is measured after transmission.
Plasmid carriers (p_1)	Carries a trait which provides benefit *B* to all individuals within the same deme (*j*) coming at a cost *C* to the cooperative individual (*i*)
Resistance/suppressor carriers (p_2)	Resistance model: Carries resistance to infection by plasmids Suppressor model: Carries an inhibitor which prevents expression of the plasmids cooperative behaviour
N	Number of founder strains on each patch
n	Number of patches in the population
β	Transmission probability of the plasmid
C	Cost of cooperative behaviour to the cooperative individual (*i*)
v	Baseline cost of plasmid carriage
C_1	Cost of cooperative behaviour to the cooperative individual (*i*) plus baseline cost of plasmid carriage i.e. $C_1=C+v$

C_2	Resistance model: Cost of expressing resistance Suppressor model: Cost of expressing supressor
h	Suppressor model: effect of suppressor on cooperative gene expression and its associated costs and benefits i.e. h=1 gives full suppression of the cooperative trait and h=0 gives no suppression (i.e. full expression) of the cooperative gene
B	Benefit of cooperative behaviour. Shared by all individuals within the same deme (j)

Rearranging equation (1), as described in Appendix A, and applying the fitness term above gives the change, over one generation, in plasmid and resistance frequency from one generation to the next in p_x, in terms of p_x^{t}:

$$\Delta p_1 = p_1^{\mathrm{t}}(1-p_1^{\mathrm{t}})\left(\frac{BR-C_1}{1+Bp_1^{\mathrm{t}}-C_1p_1^{\mathrm{t}}-C_2p_2^{\mathrm{t}}}\right)+p_1^{\mathrm{t}}p_2^{\mathrm{t}}\left(\frac{C_2}{1+Bp_1^{\mathrm{t}}-C_1p_1^{\mathrm{t}}-C_2p_2^{\mathrm{t}}}\right)+\Delta p_1^{t} \qquad [3a]$$

$$\Delta p_2 = \frac{1}{w}\left(p_1^{\mathrm{t}}p_2^{\mathrm{t}}\left(BQ_{12}+C_1\right)-C_2p_2^{\mathrm{t}}(1-p_2^{\mathrm{t}})\right) \qquad [3b]$$

Where $\Delta p_1^{\,t} = p_1^{\,t} - p_1 = (1-p_1-p_2)\frac{N-1}{N}\beta p_1$; R refers to whole group relatedness with respect to the plasmid, measured after transmission ($R = \mathrm{Cov}[p_{1j}^{\mathrm{t}}\, p_{1j}^{\mathrm{t}}]$); and Q_{12} refers to the within-patch association between plasmid carriers and resistance cells ($Q_{12} = \mathrm{Cov}[p_{1j}^{\mathrm{t}}\, p_{2j}^{\mathrm{t}}]$), also measured after transmission (see Appendix A for details).

Result 1: Plasmid cooperation leads to conflict with the chromosome

From equation (2a), we find that, in the absence of transmission, the cooperative plasmid may spread provided $B\frac{1}{N} > C_1$, when both the plasmid and the resistance gene are rare. This is essentially a version of Hamilton's rule $BR > C$ where R=1/N refers to "whole group relatedness" (Pepper 2000). If the plasmid can transmit horizontally (i.e. if β>0), a cooperative plasmid will spread from rare (when the resistance gene is also rare) under less stringent conditions (where $C_1 = C + v$):

$$B\left(\frac{1}{N}+\beta\frac{N-1}{N}\frac{1}{N}\right)+\beta\frac{N-1}{N+\beta(N-1)} > C+v\,. \qquad [4]$$

It is clear that, if $C+v>B/N$, but inequality (4) still holds, there will be a conflict between a cooperative gene carried on a plasmid, with the bacterial chromosome. Fig. 1 illustrates this conflict between the chromosome and the plasmid, where the cooperative behaviour is favoured by spreading horizontally in scenarios where the trait would not be favoured if it were chromosomally based. This conflict is driven by the two reasons why cooperative genes are favoured on plasmids (Rankin et al. 2011a): the first is due to infectivity, where the plasmid simply spreads to previously uninfected hosts (which, when the plasmid is rare, is given by the term $\beta(N-1)/(N+\beta(N-1))$ in inequality (4)), while the other is due to an amplification of local genetic similarity in a patch (which, when the plasmid is rare, is given by the term $\beta(N-1)/N^2$ in equality (4)).

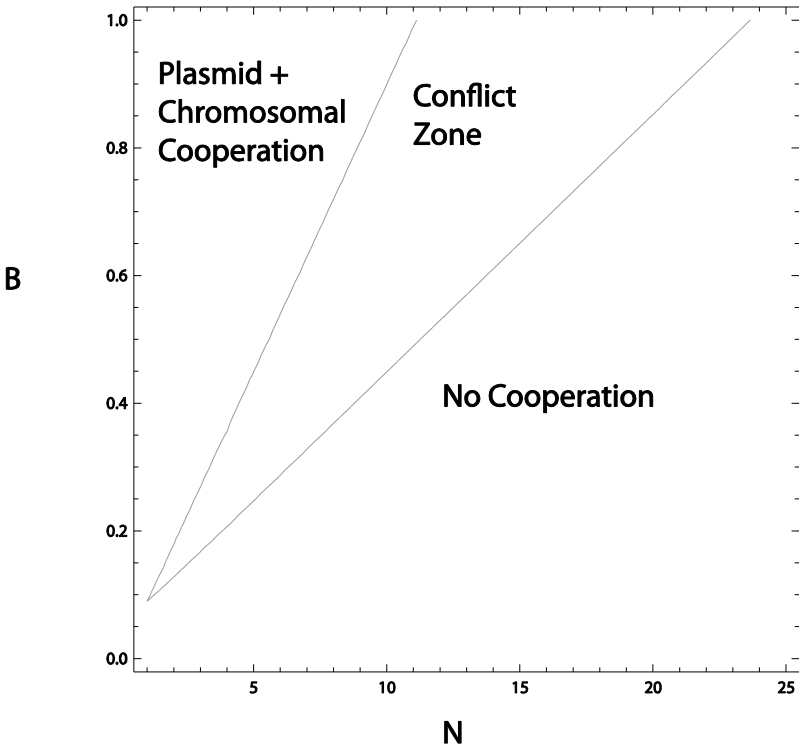

Figure 1. Conflict between the host chromosome and the plasmid with respect to cooperative traits

The areas favouring each scenario are denoted. The area where it is only in the plasmids interest for the cell to cooperate, but not for the chromosome, can be seen as the "conflict zone". Chromosomal cooperation is favoured when $B\frac{1}{N} > C_1$, whereas plasmid-based cooperation is favoured when $B\left(\frac{1}{N}+\beta\frac{N-1}{N}\frac{1}{N}\right)+\beta\frac{N-1}{N+\beta(N-1)} > C_1$ allowing plasmid-carried cooperation to persist in areas where $B\frac{1}{N} < C_1$. Parameters: $\beta = 0.05$, $C_1 = 0.09$, $B = 0.3$.

Result 2: HGT does not affect invasion of resistance

The condition for the resistance gene to invade a population where the cooperative gene is at fixation when there is no HGT (i.e. when β=0) is $C_1 - B/N < C_2$. This shows that resistance evolves independently of the degree of HGT (β>0) as the resistance mechanism blocks the transmission of the plasmid into uninfected cells, and thus removes the advantage of horizontal transfer. However, the cooperative gene will only invade if it is on a plasmid if β>0 when C_1>B/N (from inequality (4)).

Result 3: Full resistance frequently leads to cyclical dynamics

Fig. 2A shows the dynamics of the plasmid versus the resistance gene. We use a parameter set that allows coexistence and we see that this coexistence displays cyclical dynamics suggesting that this mechanism of conflict resolution will frequently be unstable and will result in cycles between resistant cells, plasmid-infected cells and cells which carry neither trait.

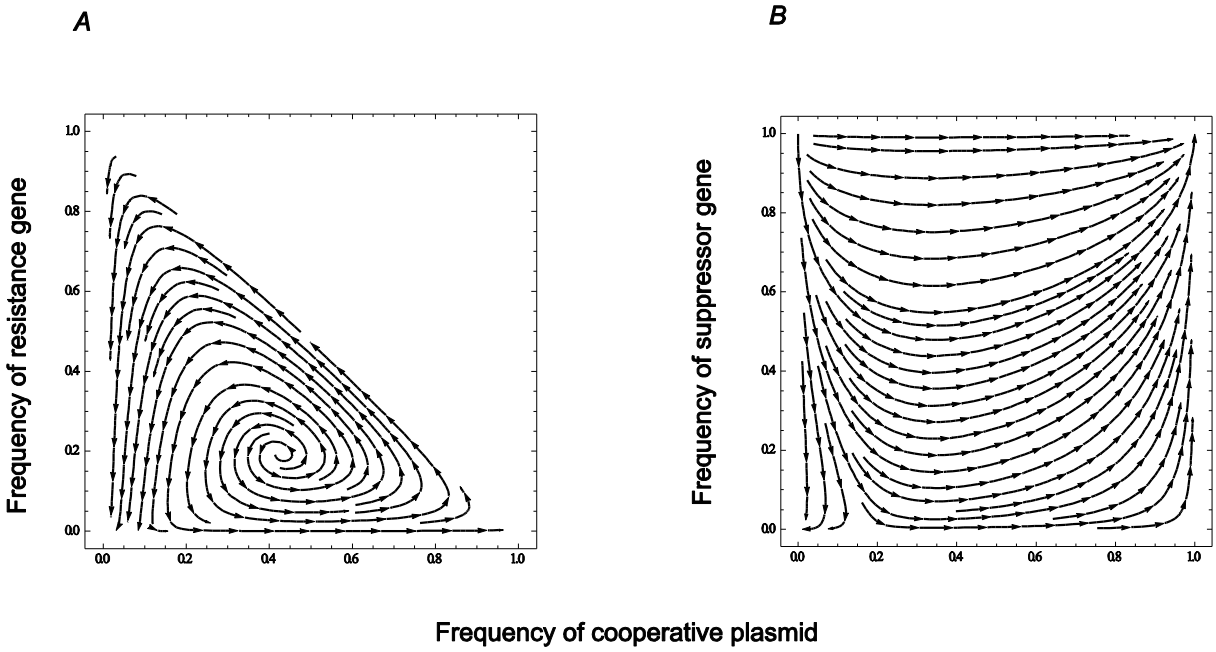

Figure 2. Dynamics of two mechanisms of defence against plasmids.

Parameters: β = 0.2, B = 0.4, N = 100. For parameter values where there is coexistence between the resistance mechanism and the plasmid full resistance results in cyclical dynamics whereas in contrast both traits can go to fixation for the suppressor model. Panel (a) model (1)

resistance to plasmids, $C_1 = 0.18$, $C_2 = 0.05$. Panel (b) model (2) suppression of cooperative behaviour, $v = 0.05$, $C_1 = 0.13$, $C_2 = 0.05$, h=1.

Model 2 – Chromosomal suppression of plasmid gene expression

In our previous model, resistance against the plasmid acted by preventing infection of a cell with the plasmid. Here we focus on an alternative method, namely suppression of the plasmid-carried cooperative gene, which targets only the social trait carried on the plasmid not the plasmid itself. We assume that a suppressor carried on the host chromosome can act to decrease/fully suppress expression of specific plasmid genes (it may, of course, also function to enhance plasmid gene expression but we focus here on suppression in order to decrease the conflict between chromosome and plasmid). We assume that the plasmid can still transfer to plasmid-free cells by conjugation, regardless of whether the cell carries the suppressor allele. Thus, cells carrying the suppressor can be infected with the plasmid but their expression of the plasmid-borne cooperative trait is reduced (see below). The global frequency of plasmid carriers is denoted by p_1, while p_2 now refers to the global frequency of cells carrying the suppressor trait. The fitness function for this model is:

$$w_{ij} = 1 + Bp_{1j}{}^{t}\left(1 - hp_{2j}{}^{t}\right) - Cp_{1ij}{}^{t}\left(1 - hCp_{2ij}{}^{t}\right) - C_2 p_{2ij}{}^{t} - vp_{1ij}{}^{t}. \qquad [5]$$

Here C refers solely to the cost of expressing the cooperative gene and the cost of plasmid-carriage is denoted by v (this is not affected by the suppressor gene). The suppressor affects expression of the cooperative trait in cells which carry both the suppressor and the plasmid. The suppressor's effect on the costs and benefits associated with the expression of the cooperative trait (C and B) is controlled by parameter h (where h=0 results in full expression of the cooperative trait and h=1 in full inhibition of the cooperative trait by the suppressor). $p_1{}^{t}$ is the sum of those cells who initially carried the plasmid plus those plasmid-free cells which were infected with the plasmid at the transmission stage (regardless of whether or not they carried the suppressor gene) such that $p_1{}^{t} = p_1 + (1 - p_1)\frac{N-1}{N}\beta p_1$. As in the previous model, p2 is not transmitted horizontally and therefore $p_2{}^{t} = p_2$. As before, the terms used to calculate fitness are listed in Table 1.

As before, we can use the Price Equation (equation (1)) to describe the changes in the frequency of the suppressor and the plasmid from one generation to the next as (see Appendix B for details):

$$\Delta p_1 = \frac{p_1^t(1-p_1^t)\left(BR - C - v - p_2^t(BR-C)h\right)}{1 + Bp_1^t\left(1-hp_2^t\right) - Cp_1^t\left(1-hp_2^t\right) - C_2 p_2^t - vp_1^t} + \Delta p_1^t \quad [6a]$$

$$\Delta p_2 = \frac{\left(p_1{}^t p_2{}^t\left(B(Q_{12} - hp_2{}^t Q_{23}) + hC(1-p_2{}^t)\right) - C_2 p_2{}^t(1-p_2{}^t)\right)}{1 + Bp_1{}^t\left(1-hp_{2_j}{}^t\right) - Cp_1{}^t\left(1-hp_2{}^t\right) - C_2 p_2{}^t - vp_1{}^t}, \quad [6b]$$

where $\Delta p_1{}^t = (1-p_1)\frac{N-1}{N}\beta p_1$; R again refers to whole group relatedness with respect to the plasmid; Q_{12} refers to the association between the plasmid carriers and suppressor carriers; and Q_{23} refers to the association between suppressor carriers and cells which carry both the suppressor and the plasmid (where individuals who carry both alleles are denoted by the subscript "3") - see Appendix B for details.

Result 4: Different mechanisms of conflict resolution show similar invasion criteria

As before, the plasmid can spread in the absence of both transmission and the conflict resolution mechanism (in this case the suppressor gene) if $BR > C + v$ (where whole-group relatedness is given by $R = \frac{1}{N}$). As we assume a cost to the suppressor gene, it cannot spread to fixation in the population in the absence of the plasmid (as we assume that C_2>0). The suppressor gene can spread from rare when the plasmid is at fixation if

$$hC > C_2 + hB\frac{1}{N},$$

which requires that $1 + B > C + v$. This condition is similar to that of the resistance gene invading a population fixed for plasmids ($C_1 > \frac{1}{N}B + C_2$).

Result 5: Horizontal gene transfer remains advantageous for invasion when the suppressor is at fixation

When the suppressor gene is at fixation, the cooperative plasmid cannot invade from rare in the absence of HGT. However, in contrast to model 1, in the presence of HGT, the plasmid can invade the suppressor at fixation (i.e. if $p_2 \rightarrow 1$and h=1), provided $(N-1/N)\beta > x/(C_2 + x - 1)$and C_2<1. While the suppressor controls the expression of the cooperative trait (such that the host cell is no longer at the mercy of the plasmid's social behaviour), it does not impact on the transfer of the plasmid.

Result 6: Suppression of cooperative genes is a more stable form of defense against plasmids than full resistance

Fig. 2B shows that the plasmid and the suppressor gene can lead to stable coexistence. However, we do not observe cycling between the suppressor gene and the plasmid, as we did in model 1 (see Result 3). The suppressor mechanism allows for plasmid carriage, and simply inhibits expression of the genes carried on the plasmid, while "chromosomal resistance" (i.e. prevention of carriage of the cooperative gene altogether) does not allow for a plasmid to be carried in a cell carrying the resistance gene. Thus, chromosomal resistance can be seen as analogous to host-parasite or predator-prey interactions, where the resistance gene has a strong impact on the fitness of the plasmid, and thus leads to intransitive dynamics. Thus full resistance (model 1) leads to cycles of plasmid-carriers, resistance-carriers and empty cells (model 1), while suppression (model 2) allows for stable coexistence with both the plasmid and the suppression gene being able to go to fixation.

Discussion

Our models investigate the coevolution between plasmids and the host chromosome, specifically when plasmids carry genes coding for a cooperative traits. Plasmids can be seen as selfish genetic elements, which replicate independently of the host chromosome, and our study has examined the mechanisms by which bacterial chromosomes can mitigate the costs of carrying such selfish mobile elements. In our model, the mitigation of costs occurs either by resisting plasmid infection or by regulating certain aspects of plasmid gene expression. In the case of chromosomal resistance, we find that the coevolutionary dynamics of plasmid carriage and chromosomal resistance to a plasmid frequently results in non-transitive cycling between plasmids, resistance genes and wild-type cells (i.e. cells without either the plasmid or the resistance gene – Figure 2). These non-transitive dynamics reflect rock-paper-scissors dynamics (Sinervo and Lively 1996; Kerr et al. 2002), a common phenomenon in coevolutionary interactions. We do not observe cycling in our model incorporating a suppressor mechanism (due to the fact that a cell can simultaneously carry both the plasmid and the chromosomal suppressor gene leading to stable coexistence), suggesting that resistance is likely to be less stable than suppression of plasmid genes as a mechanism to resolve the conflict between a plasmid and the bacterial chromosome.

Genomic conflict is prevalent at all levels of biological organization (e.g. Keller 1999) and here we examine the existence of a conflict between a host chromosome and its plasmid with respect to cooperative traits, first highlighting the region where conflict will occur (Figure 1). Plasmids have been previously suggested as a strategy for a gene to impose a particular phenotype on cells (Smith 2001): the ability to force a host to express given genes can result in a cell performing certain behaviours (e.g. cooperation) in a way that is suboptimal for the chromosome, but beneficial for the plasmid carrying such a gene (as seen here, when plasmid-carried cooperation is favoured even though $0<BR<C$). Horizontal gene transfer can thus help to promote the spread of genes that would not otherwise be favoured in the absence of horizontal transmission (Smith 2001; Nogueira et al. 2009; Mc Ginty et al. 2011). Horizontal gene transfer itself can therefore drive conflict between the chromosome and the plasmid (Figure 1).

Full resistance may appear to be the most effective way to deal with such genes because it completely removes their advantage of horizontal spread whilst also negating the cost of plasmid carriage for the host. However stable coexistence between the plasmid and the resistance gene is not possible and resistance frequently results in non-transitive cycling. Most

forms of resistance in host-parasite systems involve costs to the host (Sheldon and Verhulst 1996), which determine both the resulting co-evolutionary dynamics and the extent to which there is variance in the effects of different resistance genes (Antonovics and Thrall 1994). Although measurements of costs of resistance are limited (Lennon et al. 2007), in the case of CRISPRs, it is likely that the costs of resistance will be associated with the length of, or the number of, CRISPRs in the genome (Vale and Little 2010).

Our second model involves a host-expressed suppressor, which reduces (or entirely prevents) the expression of the plasmid-carried gene (here, a social trait). In contrast to the full resistance model, gene suppression does not exhibit cycling, primarily because plasmids maintain the ability to propagate. It is possible that gene regulation mechanisms may inflict lower costs than complete resistance: for example a mechanism such as methylation is likely to be relatively low-cost as it is a common feature of cell development (Jaenisch and Bird 2003). However, gene regulation is not without its drawbacks. Conflict arises both from the carriage of the plasmid but also from the expression of its genes. Therefore the advantage of gene regulation as a mechanism to counter the costs of plasmid infection, over full resistance, will depend upon the costs of plasmid carriage and the net cost of expressing genes carried on a plasmid. While we have not looked at competition between resistance and suppression directly, we would expect that resistance will be more likely to evolve if there is a higher cost to plasmid carriage (i.e. if v is high), as opposed to suppression, which will evolve if there is a higher net cost of expressing genes on the plasmid (i.e. if C is high, and $C>BR$, even if the costs v from plasmid carriage are high).

Our results highlight the causes and consequences of coevolution between plasmids and their bacterial hosts. Bacteria have previously been shown to adapt to the presence of plasmids (Bouma and Lenski 1988; Modi and Adams 1991), and plasmids themselves may also adapt to the host (Modi and Adams 1991). In our model, the host chromosome evolves a given mechanism to mitigate the cost of plasmid carriage, whether that is resistance (in model 1 – the "resistance" model) or suppression of plasmid gene expression (in model 2 – the "suppression" model). In the case of the resistance mechanism, it is possible that the plasmid may evolve to counter the influence of resistance, a phenomenon that has been observed to occur in CRISPR systems (Semenova et al. 2011). In contrast to an outright resistance mechanism, a gene regulation mechanism may be more stable because it still allows the plasmid to propagate, and thus has the potential to reduce the selective pressure for plasmid counter-adaptations. It is thus possible that coevolution between the plasmid and the host itself may mitigate the cost caused by carriage of the plasmid by reducing the cost of plasmid carriage without the need to develop costly systems such as CRISPRs (Bouma and Lenski 1988; Modi and Adams 1991).

There are other ways in which the costs of plasmid carried cooperation can be reduced, which we have not considered in our model. For example, the reduction of the number of plasmids carried by a host (i.e. the copy number of the plasmid – Iordanescu and Bargonetti 1989) or an increase in the rate of plasmid segregation (e.g. Modi and Adams 1991) could reduce the costs imposed by a plasmid on the host cell. Large plasmids are suggested to be the origin of bacterial secondary chromosomes (Smillie et al. 2010), as has been shown in *Rhizobiaceae* (Slater et al. 2009). Conversion of a plasmid to a secondary chromosome may be viewed as a

potential mechanism of conflict resolution where the ability of a plasmid to transfer to other hosts would be reduced, for example by their own fertility inhibition systems (Dionisio et al. 2002; Haft et al. 2009), which would then help to align the interests of the plasmid and the host chromosome (Dahlberg and Chao 2003).

Our model has shown that, when a plasmid carries genes involved in cooperation, there is the potential for a conflict between the plasmid and the host chromosome. These results suggest that the mechanisms that mitigate the costs of plasmid gene expression will generally be more stable, than full resistance mechanisms. Our model bears similarities to other conflict resolving mechanisms such as policing in social insects (e.g. Foster and Ratnieks 2000), where "suppression" would be seen as analogous to the "policing" which act to the detriment of the colony. In policing, reproduction by workers is repressed by policing individuals, for the good of the colony (e.g. Foster and Ratnieks 2000). The wider implication is that "policing", or suppression of genes already within a cell, is a more stable option for a host cell to resolve a genetic conflict than actively inhibiting the spread of the mobile element. However, as a gene regulation mechanism merely targets gene products and not the plasmid itself, only full resistance mechanisms can eliminate conflict, however briefly, between plasmids, and their bacterial hosts.

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References

Ahmer, B. M. M., M. Tran, and F. Heffron. 1999. The virulence plasmid of Salmonella typhimurium is self-transmissible. Journal of Bacteriology 181:1364-1368.

Antonovics, J., and P. H. Thrall. 1994. The Cost of Resistance and the Maintenance of Genetic Polymorphism in Host-Pathogen Systems. Proceedings of the Royal Society of London. Series B: Biological Sciences 257:105-110.

Bouma, J. E., and R. E. Lenski. 1988. Evolution of a Bacteria Plasmid Association. Nature 335:351-352.

Burt, A., and R. Trivers. 2008. Genes in Conflict: The Biology of Selfish Genetic Elements. Harvard University Press.

Cámara, B., M. Liu, J. Reynolds, A. Shadrin, B. Liu, K. Kwok, P. Simpson, R. Weinzierl, K. Severinov, E. Cota, S. Matthews, and S. R. Wigneshweraraj. 2010. T7 phage protein Gp2 inhibits the Escherichia coli RNA polymerase by antagonizing stable DNA strand separation near the transcription start site. Proceedings of the National Academy of Sciences 107:2247-2252.

Chernin, L. S., and V. S. Mikoyan. 1981. Effects of plasmids on chromosome metabolism in bacteria. Plasmid 6:119-140.

Close, T. J., R. C. Tait, and C. I. Kado. 1985. Regulation of Ti plasmid virulence genes by a chromosomal locus of Agrobacterium tumefaciens. J. Bacteriol. 164:774-781.

Dahlberg, C., and L. Chao. 2003. Amelioration of the Cost of Conjugative Plasmid Carriage in Eschericha coli K12. Genetics 165:1641-1649.

Dionisio, F., I. Matic, M. Radman, O. R. Rodrigues, and F. Taddei. 2002. Plasmids Spread Very Fast in Heterogeneous Bacterial Communities. Genetics 162:1525-1532.

Ellis, R. J., A. K. Lilley, S. J. Lacey, D. Murrell, and H. C. J. Godfray. 2007. Frequency-dependent advantages of plasmid carriage by Pseudomonas in homogeneous and spatially structured environments. Isme J. 1:92-95.
Foster, K. R., and F. L. W. Ratnieks. 2000. Social insects: Facultative worker policing in a wasp. Nature 407:692-693.
Frank, S. A. 1998. Foundations of social evolution. Princeton University Press, Princeton N.J.
Gardner, A., S. A. West, and A. Buckling. 2004. Bacteriocins, spite and virulence. Proceedings of the Royal Society of London Series B-Biological Sciences 271:1529-1535.
Gonzalez, J. E., and M. M. Marketon. 2003. Quorum Sensing in Nitrogen-Fixing Rhizobia. Microbiol. Mol. Biol. Rev. 67:574-592.
Gordo, I., and A. Sousa. 2010. Mutation, Selection and Genetic Interactions in Bacteria. John Wiley & Sons, Ltd, Chichester.
Griffin, A. S., S. A. West, and A. Buckling. 2004. Cooperation and competition in pathogenic bacteria. Nature 430:1024-1027.
Haft, R. J. F., J. E. Mittler, and B. Traxler. 2009. Competition favours reduced cost of plasmids to host bacteria. ISME J 3:761-769.
Hamilton, W. D. 1964. Genetical Evolution of Social Behaviour I & II. Journal of Theoretical Biology 7:1-52.
Harr, B., and C. Schlotterer. 2006. Gene expression analysis indicates extensive genotype-specific crosstalk between the conjugative F-plasmid and the E. coli chromosome. BMC Microbiology 6:80.
Hayes, F. 2003. Toxins-Antitoxins: Plasmid Maintenance, Programmed Cell Death, and Cell Cycle Arrest. Science 301:1496-1499.
Holcik, M., and V. M. Iyer. 1997. Conditionally lethal genes associated with bacterial plasmids. Microbiology 143:3403-3416.
Iordanescu, S., and J. Bargonetti. 1989. Staphylococcus aureus Chromosomal Mutations That Decrease Efficiency of Rep Utilization in Replication of pT181 and Related Plasmids. Journal of Bacteriology 171:4501-4503.
Jaenisch, R., and A. Bird. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nature Genetics 33:245-254.
Jensen, R. B., and K. Gerdes. 1995. Programmed cell death in bacteria: proteic plasmid stabilization systems. Molecular Microbiology 17:205-210.
Johnson, L. J. 2007. The genome strikes back: The evolutionary importance of defence against mobile elements. Evol. Biol. 34:121-129.
Keller, L. 1999. Levels of selection in evolution. Princeton University Press, Chichester.
Kerr, B., M. A. Riley, M. W. Feldman, and B. J. M. Bohannan. 2002. Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. Nature 418:171-174.
Lee, K., M. Lee, J. H. Shin, M. H. Lee, S. H. Kang, A. J. Park, D. Yong, Y. Chong, and K. Participants. 2006. Prevalence of plasmid-mediated AmpC beta-lactamases in Escherichia coli and Klebsiella pneumoniae in Korea. Microbial Drug Resistance-Mechanisms Epidemiology and Disease 12:44-49.
Lennon, J. T., S. A. M. Khatana, M. F. Marston, and J. B. H. Martiny. 2007. Is there a cost of virus resistance in marine cyanobacteria? Isme J. 1:300-312.
Levin, B. R. 1993. The accessory genetic elements of bacteria: existence conditions and (co)evolution. Current Opinion in Genetics & Development 3:849-854.
Lili, L. N., N. F. Britton, and E. J. Feil. 2007. The persistence of parasitic plasmids. Genetics 177:399-405.
Mc Ginty, S. E., D. J. Rankin, and S. P. Brown. 2011. Horizontal Gene Transfer and the Evolution of Bacterial Cooperation. Evolution 65:21-32.

Modi, R. I., and J. Adams. 1991. Coevolution in Bacterial-Plasmid Populations. Evolution 45:656-667.
Nogueira, T., D. J. Rankin, M. Touchon, F. Taddei, S. P. Brown, and E. P. C. Rocha. 2009. Horizontal Gene Transfer of the Secretome Drives the Evolution of Bacterial Cooperation and Virulence. Current biology : CB 19:1683-1691.
Novick, R. P. 1987. Plasmid Incompatibility. Microbiological Reviews 51:381-395.
Penalver, C. G. N., F. Cantet, D. Morin, D. Haras, and J. A. Vorholt. 2006. A Plasmid-Borne Truncated luxI Homolog Controls Quorum-Sensing Systems and Extracellular Carbohydrate Production in Methylobacterium extorquens AM1. J. Bacteriol. 188:7321-7324.
Pepper, J. W. 2000. Relatedness in Trait Group Models of Social Evolution. Journal of Theoretical Biology 206:355-368.
Price, G. R. 1970. Selection and Covariance. Nature 227:520-521.
Price, G. R. 1972. Extension of covariance selection mathematics. Annals of Human Genetics 35:485-490.
Rankin, D. J., S. E. Mc Ginty, T. Nogueira, M. Touchon, F. Taddei, E. P. C. Rocha, and S. P. Brown. 2011a. Bacterial cooperation controlled by mobile elements: kin selection and infectivity are part of the same process. Heredity 107:279-281.
Rankin, D. J., E. P. C. Rocha, and S. P. Brown. 2011b. What traits are carried on mobile genetic elements, and why? Heredity 106:1-10.
Schumann, W. 2001. The biology of plasmids *in* M. Schleef, ed. Plasmids for therapy and vaccination Wiley-VCH, Weinheim ; Chichester
Semenova, E., M. M. Jore, K. A. Datsenko, A. Semenova, E. R. Westra, B. Wanner, J. van der Oost, S. J. J. Brouns, and K. Severinov. 2011. Interference by clustered regularly interspaced short palindromic repeat (CRISPR) RNA is governed by a seed sequence. Proceedings of the National Academy of Sciences.
Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution 11:317-321.
Shintani, M., Y. Takahashi, H. Tokumaru, K. Kadota, H. Hara, M. Miyakoshi, K. Naito, H. Yamane, H. Nishida, and H. Nojiri. 2010. Response of the Pseudomonas host chromosomal transcriptome to carriage of the IncP-7 plasmid pCAR1. Environmental Microbiology 12:1413-1426.
Simonsen, L. 1991. The Existence Conditions for Bacterial Plasmids: Theory and Reality. Microbial Ecology 22:187-205.
Sinervo, B., and C. M. Lively. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. Nature 380:240-243.
Slater, S. C., B. S. Goldman, B. Goodner, J. C. Setubal, S. K. Farrand, E. W. Nester, T. J. Burr, L. Banta, A. W. Dickerman, I. Paulsen, L. Otten, G. Suen, R. Welch, N. F. Almeida, F. Arnold, O. T. Burton, Z. Du, A. Ewing, E. Godsy, S. Heisel, K. L. Houmiel, J. Jhaveri, J. Lu, N. M. Miller, S. Norton, Q. Chen, W. Phoolcharoen, V. Ohlin, D. Ondrusek, N. Pride, S. L. Stricklin, J. Sun, C. Wheeler, L. Wilson, H. Zhu, and D. W. Wood. 2009. Genome Sequences of Three Agrobacterium Biovars Help Elucidate the Evolution of Multichromosome Genomes in Bacteria. J. Bacteriol. 191:2501-2511.
Smillie, C., M. P. Garcillan-Barcia, M. V. Francia, E. P. C. Rocha, and F. de la Cruz. 2010. Mobility of Plasmids. Microbiology and Molecular Biology Reviews 74:434-352.
Smith, J. 2001. The social evolution of bacterial pathogenesis. Proceedings of the Royal Society of London Series B-Biological Sciences 268:61-69.
Sorek, R., V. Kunin, and P. Hugenholtz. 2008. CRISPR - a widespread system that provides acquired resistance against phages in bacteria and archaea. Nature Reviews Microbiology 6:181-186.

Sota, M., and E. M. Top. 2008. Horizontal gene transfer mediated by plasmids *in* G. Lipps, ed. Plasmids: Current Research and Future Trends Caister Academic Press, Norfolk.
Stern, A., and R. Sorek. 2011. The phage-host arms race: Shaping the evolution of microbes. Bioessays 33:43-51.
Taylor, P. D., and S. A. Frank. 1996. How to make a kin selection model. Journal of Theoretical Biology 180:27-37.
Vale, P. F., and T. J. Little. 2010. CRISPR-mediated phage resistance and the ghost of coevolution past. Proceedings of the Royal Society B-Biological Sciences 277:2097-2103.
van der Oost, J., M. M. Jore, E. R. Westra, M. Lundgren, and S. J. J. Brouns. 2009. CRISPR-based adaptive and heritable immunity in prokaryotes. Trends in Biochemical Sciences 34:401-407.
Wagner, E. K., and M. J. Hewlett. 2004. Basic Virology. Blackwell Science, Oxford.
West, S. A., and A. Buckling. 2003. Cooperation, virulence and siderophore production in bacterial parasites. Proceedings of the Royal Society of London Series B-Biological Sciences 270:37-44.
Wilson, G. G., and N. E. Murray. 1991. Restriction and Modification Systems. Annu. Rev. Genet. 25:585-627.